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High Gradient Magnetic Cell Separation With MACS[1]

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A flexible, fast and simple magnetic cell sorting system for separation of large numbers of cells according to specific cell surface markers was developed and tested. Cells stained sequentially with biotinylated antibodies, fluorochrome-conjugated avidin, and superparamagnetic biotinylated-microparticles (about 100 nm diameter) are separated on high gradient magnetic (HGM) columns. Unlabelled cells pass through the column, while labelled cells are retained. The retained cells can be easily eluted. More than 10^9 cells can be processed in about 15 min. Enrichment rates of more than 100-fold and depletion rates of several 1,000-fold can be achieved. The simultaneous tagging of cells with fluorochromes and very small, invisible magnetic beads makes this system an ideal complement to flow cytometry. Light scatter and fluorescent parameters of the cells are not changed by the bound particles. Magnetically separated cells can be analysed by fluorescence microscopy or flow cytometry or sorted by fluorescence-activated cell sorting without further treatment. Magnetic tagging and separation does not affect cell viability and proliferation.

Key terms: Superparamagnetic microbeads, magneto-immunofluorescent labelling, rare-cell isolation

Fluorescence activated cell sorting (FACS, 18) is a powerful method for isolation of subpopulations out of complex cell mixtures, but application is often limited by the relatively small separation capacity of approximately 10^7 particles per hour. Isolation of rare cells is time-consuming and laborious. Processing of large numbers of cells, e.g., for molecular analysis or clinical application, in many cases is not possible. With conventional alternative immunological cell separation techniques, such as panning (8), complement lysis (7), or rosetting (2) the cells are sorted in parallel and thus much larger cell numbers can be processed. However, these methods have various serious disadvantages, including low sensitivity, poor quality of separation, loss of the labelled cells, and amount of reagent and time required. Moreover, such separations are hard to control. Most magnetic cell separation techniques described so far (1,3,11; for a review see 5,15) resemble panning and rosetting in many aspects.

Cells labelled immunologically with large magnetic particles ($\text{diameter}_{\text{particle}} \geq 0.5$ μm) (1,3,5) can be easily separated from unlabelled cells by a simple permanent magnet. Although conferring a large magnetic moment on the labelled cells, large magnetic particles have major disadvantages. Usually they are useful only for depletion of cells. Bound large particles interfere with cell viability and bound cells are difficult to detach because of the multiple-point attachment. They change the optical properties of the labelled cells. Large magnetic particles aggregate because they are too magnetic and cells get trapped nonspecifically in the aggregates.

Magnetic microparticles ($\text{diameter}_{\text{particle}} \ll 0.5$ μm) can have a variety of superior characteristics compared to larger particles. The binding reaction is much faster, the cells can be labelled quantitatively, and their optical parameters are not changed, the beads do not aggregate, not even in a magnetic field, and are easy to sterilize. Their major disadvantage is the small magnetic moment, resulting in long separation times in magnetic fields of conventional geometries (6,11).

To overcome this disadvantage, Molday and Molday have suggested a combination of small superparamagnetic microparticles and high gradient magnetic (HGM) fields (13). We have worked out and tested a cell separation method according to this concept and added fluorescent labelling to the process. The resulting preparative magnetic cell separation system (MACS) allows efficient enrichment as well as depletion of la-

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belled cells. Fluorescence microscopy or flow cytometry are useful for direct control of the magnetic sort. Also, MACS is ideally suited for preenrichment of rare cells according to one parameter for multiparameter fluorescence activated cell sorting.

MATERIAL AND METHODS

Magnetic Microparticles

Magnetic microparticles were produced by a modification of the procedure of Molday and MacKenzie (12). Ten grams of Dextran T40 (Pharmacia Uppsala, Sweden), 1.5 g $FeCl_3 \cdot 6\ H_2O$ and 0.64 g $FeCl_2 \cdot 4\ H_2O$ are dissolved in 20 ml H_2O, and heated to 40°C. While stirring, 10 ml 4N NaOH are added slowly and the solution is heated to 70°c for 5 min. The particle suspension is neutralised with acetic acid. To remove aggregates the suspension is centrifuged for 10 min at 2,000*g* and filtrated through a 0.22 μm pore-size filter (Millex GV, Millipore, Molsheim, France). Unbound Dextran is removed by washing in a high-gradient magnetic field (HGMF). HGMF washing of magnetic particles is performed in steelwool columns made as described below and placed in a magnetic field of approx. 0.6 Tesla (MACS permanent magnet, Miltenyi Biotec GmbH, Bergisch Gladbach, West Germany; or laboratory electromagnet). Ten milliliters of particle suspension are applied to a 15 × 40 mm column of 2 g steelwool. The loaded column is washed with 30 ml 0.05 M sodium-acetate. After removing the column from the external magnetic field, the magnetic particles are eluted with 0.05 M sodium-acetate.

The particles form a brown suspension, which is stable for more than 12 months at 4°C. The relative particle concentration is given as optical density at 450 nm. The size of the beads was determined by electron microscopy and dynamic light scattering (10) to be 30 ± 20nm (e.m.) and 65 ± 20 nm (DLS). The beads show superparamagnetic behavior, as determined by susceptibility measurements. The size of the trapped ferrit-microcrystals was determined from magnetic measurements to be approximately 10 nm.

Biotinylation of Magnetic Microparticles

Various molecules could be coupled to the particles. Biotinylated particles are advantageous for many applications and have been used for the experiments described here.

For biotinylation of superparamagnetic particles, 10 ml of the microparticle suspension, rebuffered in 0.1 M Na_2CO_3, are activated for 10 min with 0.5 mg cyanogen-bromide in acetonitrile. One milliliter of 0.2 M diaminohexane (pH 7) is added and the mixture is titrated to pH 8.5 with 0.5 M NaH_2PO_4. After stirring for 2 h at 4°C, unconjugated diaminohexan is removed by HGM washing. The particles are resuspended in 10 ml 0.1 M $NaHCO_3$ and reacted with 10 mg biotin-succinimide (Pierce, Oud-Beijerland, Netherlands), dissolved in 200 μl DMSO. The conjugation proceeds for 8 h at room temperature, then unbound biotin is removed by HGMF and washing with phosphate-buffered saline (PBS: 0.15 M sodium-chloride, 0.01 M sodium/potassium phosphate buffer, 0.01% sodium-azide, pH 7.2). The particles are filtered through a Millex GV 0.22 μm filter and stored in sterile aliquots at 4°C at a concentration of OD_{495} = 10.

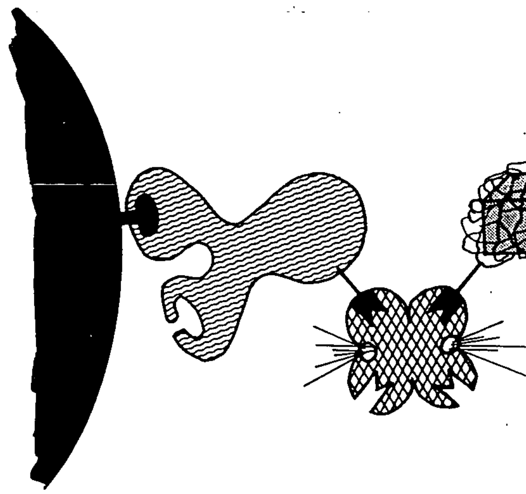

FIG. 1. Magnetofluorescence staining for MACS. Labelling of cell surface structures with biotinylated antibody, fluorochrome-conjugated streptavidin, and biotinylated superparamagnetic ferrit-dextran beads.

Cells

Spleen cells were obtained from (C57B16 × BALB/c)F_1 mice, removing red and dead cells on a Ficoll cushion (19). J558L myeloma cells and J558L-MK-F, stably transfected with the H-2 K^k gene, and the monoclonal anti-H-2K^k antibody have been described elsewhere (20). Again, live cells were purified using Ficoll(19). Erythrocytes, live and dead cells, stained with 0.5% Trypan Blue in saline, were counted in a Neubauer chamber.

Staining Procedures

We have chosen a three-step procedure for labelling of cells with biotinylated superparamagnetic beads (Fig. 1). We use the avidin-biotin system, because it is a most versatile immunological detection system. First, the cells are labelled with biotinylated antibody. If not available commercially, biotinylated antibodies are easy to prepare. First, 0.1 mg/ml of biotin-LC-hydroxysuccinimide (Pierce, from a 1 mg/ml freshly prepared stock solution in di-methylformamide) are stirred with 0.1–10 mg/ml of pure antibodies in borate buffer, pH 9.3 (dialyzed extensively, but only for the last 30 minutes at pH 9.3) for 1–2 hs. Then the mixture is dialyzed against PBS. After titration for staining, the biotinylated antibody can be stored at 4°C or, preferably, in aliquots at −70°C.

Second, the labelled cells are stained with a fluorescent avidin conjugate, e.g., fluorescein-steptavidin. Such conjugates are available commercially, e.g., from

Southern Biotechnology Assoc. (SBA, Birmingham, AL) or Becton-Dickinson (Mountain View, CA). Third, the biotinylated superparamagnetic microparticles are bound to remaining free binding sites of the avidin on the cell surface. Avidin has four biotin-binding sites. Thus, antibody binding cells finally have a fluorescent and a magnetic label.

The total staining time is about 30 min. Antibody labelling of cells is performed at 4°C for 10–15 min in PBS with 1% bovine serum albumin (BSA) and 0.01% sodium-azide. The concentration of antibody is between 1 and 200 μg/ml, as titrated beforehand. For 10^7 cells 10 μl of antibody solution are used. The cells are washed once and stained for 10–15 min at 4°C with 10 μg/ml of streptavidin-fluorescein in PBS, 20–50 μl for 10^7 cells. After one washing with PBS, the cells are labelled with microbeads, diluted ~1:1000 from the stock in PBS. After 5 min at 4°C the cells are immediately separated, or unbound particles are first removed by a single washing step. The microparticles do not sediment at gravities used for centrifugation of cells. Too long incubation may result in increased unspecific binding of particles.

Care must be taken not to introduce free biotin with the washing and staining solutions. Free biotin is present in many BSA preparations, tissue culture media, and sera and may inhibit binding of biotinylated microparticles to cell-bound avidin.

Magnetic cell sorter (MACS)

For high-gradient magnetic-field (HGMF) separation, columns of various size are used, filled to 2–4% of their volume with ferromagnetic stainless steelwool (DIN 1.4113 S, No. 0, average diameter of a fiber 50 μm; E. Haiss KG, Haslach, FRG). The surface of the steelwool is coated with plastic by immersion into laque (Miltenyi Biotec, Bergisch Gladbach, FRG) and drying, in order to avoid damage of cells due to direct contact with the steelwires and corrosion. The columns can be characterized by the product of length and packing density, termed trapping coefficient (f), and the surface of the matrix. The surface of the matrix gives an estimate for the binding capacity of a column. A column typically used for positive cell enrichment is of 1.5 × 0.6 cm size, filled with 100 mg steelwool, and has a calculated inner surface of 10 cm^2 corresponding to a capacity of ~10^7 lymphocytes bound.

The flow through the separation column is regulated by a cock and disposable needle at the outlet. Alternatively, a perfusor pump can be used.

The geometry of the HGMF-cell sorter described here is shown in Figure 2. The steelwool column is magnetized by introducing it in a magnetic field of about 0.6 Tesla. Such a magnetic field can be produced with a commercial electromagnet, or with a specially designed permanent magnet system, as shown in Figure 3 (MACS, Miltenyi Biotech GmbH, Bergisch Gladbach, FRG). The magnetized column works as an extremely sensitive filter for magnetic particles, e.g., magnetic cells. Cells labelled with superparamagnetic beads are magnetic in a magnetic field and bind to the steelwool fibers. When the column is removed from the external magnetic field, the steelwool readily demagnetizes, the superparamagnetic particles/cells are no longer bound and can be eluted as a single cell suspension.

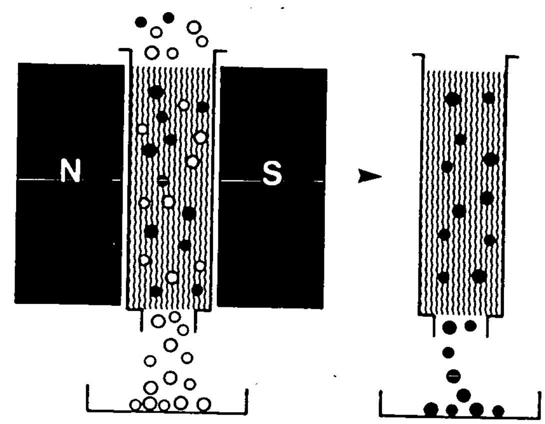

FIG. 2. High gradient magnetic cell separation. In a high gradient magnetic field, generated in a column of steelwool which is inserted into an external magnetic field, cells labelled with superparamagnetic beads will attach to the matrix. Unlabelled cells are eluted. The labelled cells can be eluted when the column is demagnetized by removal from the magnetic field.

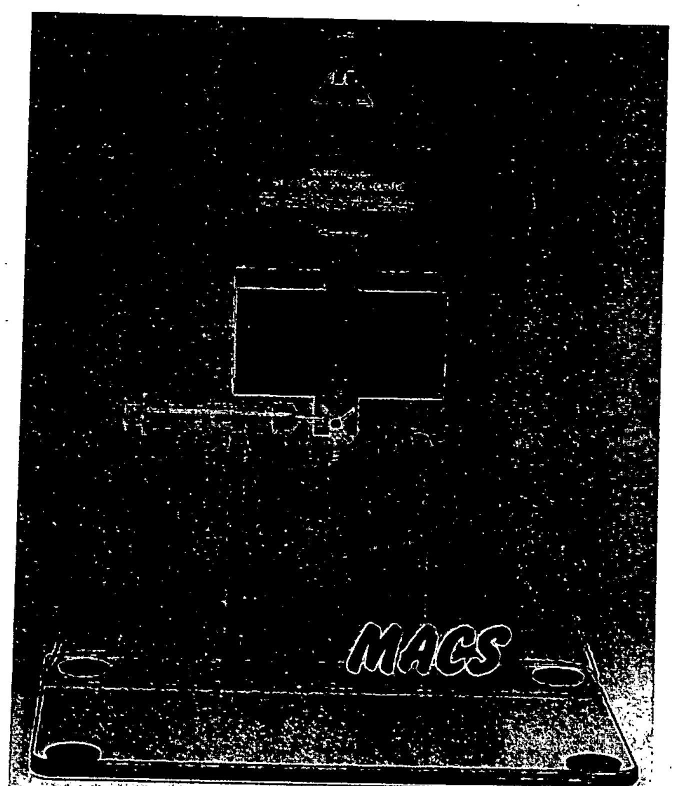

FIG. 3. The magnetic cell sorter "MACS." Photo of the MACS-system used in the experiments described here.

Magnetic Cell Sorting

The steelwool columns are stored dry at room temperature. Before use they are filled with 70% ethanol for sterilisation or with PBS either by injection from the bottom or by centrifugation. After washing with PBS, the matrix of the column is incubated with 0.5–5% BSA in PBS for 15–60 min to saturate nonspecific binding sites. The column is then flushed with ice-cold PBS/1% BSA, the labelled cells are applied, and unbound cells are washed out with 2–4 volumes of the column, or until no more cells are collected (dropwise check with an inverted microscope) at flow rates of 10–100 ml/h. Then the column is washed with more than 3 volumes PBS/1% BSA at increased flow rate. After the steelwool column is removed from the external magnetic field, the bound cells are eluted. The flow through the column is increased in order to detach all the cells bound to the matrix.

The used columns are cleaned with tissue culture detergents, rinsed with water, and dried with air pressure or suction. The columns can be used until they corrode. Packed columns are available commercially from Miltenyi Biotec GmbH (Bergisch Gladbach, FRG).

Fluorescence Microscopy

For fluorescence microscopy the cells were analysed on slides either in suspension or as cytocentrifuge smears (Shandon, Sewickley, PA), in Elvanol (Fluoromount, SBA). A Leitz Orthoplan (Leitz Wetzlar, West Germany) equipped for fluorescence microscopy was used.

Flow Cytometry

A FACS 440 or a FACScan with 488 nm argon laser excitation and collection of forward and orthogonal scatter (linear amplification) and fluorescence of propidium iodide and fluorescein (logarithmic amplification) were used (Becton-Dickinson, Mountain View, CA.). Four-parameter listmode data were recorded and analysed on a Microvax workstation (DEC, Munich, FRG) using the "electric desk" software (14) in case of the FACS 440 or a Hewlett-Packard 310 using the FACScan research software (Becton-Dickinson).

Erythrocytes, dead cells and debris were excluded by gating on forward and orthogonal scatter and/or propidium iodide.

The enrichment rate E and depletion rate 1/E are defined as

$$E = \frac{\textit{\% positive cells after sort/\% negative cells after sort}}{\textit{\% positive cells before sort/\% negative cells before sort}}$$

RESULTS

Depletion

MACS is optimized for depletion of labelled cells by using large columns with ample capacity for labelled cells and relatively slow flow rates. In Figure 4 this is demonstrated for the depletion of T lymphocytes from murine spleen cells. A total of 4×10^8 cells were labelled with biotinylated anti-Thy-1, streptavidin-fluorescein, and superparamagnetic beads. A column of 8 ml volume was used at a flow rate of ~5 cm/min (8 ml/min). The nonmagnetic, unretained fraction was collected in about 40 ml. To enumerate the negative, intermediate, and positive stained cells, two windows were set for the statistic evaluation of Thy-1 fluorescence histograms (Fig. 4). Before MACS separation 4.4% stained intermediate and 24.7% of the cells stained brightly, respectively (Fig. 4a). After sorting by MACS the unretained population contained only 0.2% intermediate and 0.01% brightly stained cells (Fig. 4b). This corresponds to depletion rates of 31- and 3,500-fold. About 70% of the unstained cells were recovered in the unretained fraction. The retained fraction eluted

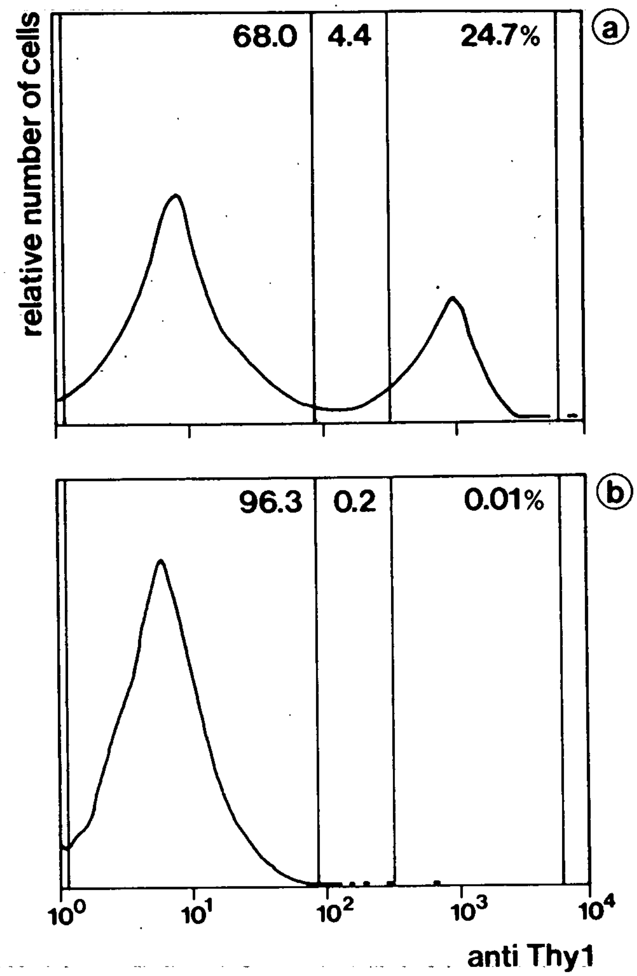

FIG. 4. Depletion of labelled cells by MACS. Fluorescence histograms of murine spleen cells, stained with a biotinylated monoclonal anti-Thy1, labelling all T lymphocytes, streptavidin-fluorescein, and superparamagnetic beads, analysed on a FACScan a) before and b) after depletion of T lymphocytes by MACS. Live cells were gated according to forward and side scatter. The histogram gives the relative number of live cells versus the logarithmically displayed relative fluorescence.

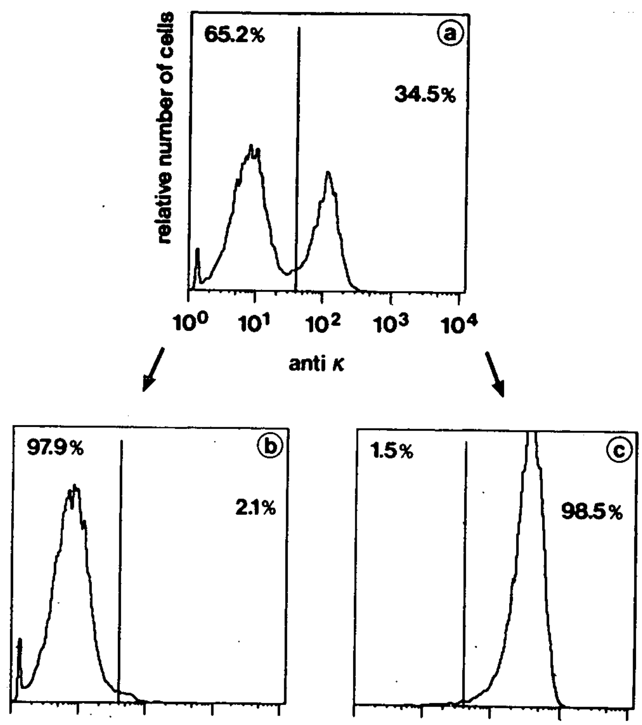

FIG. 5. MACS-separation. Fluorescence histograms of murine spleen cells, stained with a biotinylated monoclonal anti-immunoglobulin kappa light chain (anti κ) antibody, streptavidin-fluorescein, and superparamagnetic beads and separated by MACS into positive and negative subpopulations. Cells before separation (a), the unretained fraction (b), and the retained fraction (c) are analysed by FACS/EDESK and displayed as in Figure 4. Live cells had been gated according to forward and orthogonal scatter and propidium iodide fluorescence.

from the column after removal from the magnetic field was enriched for stained cells only about 3 fold (data not shown).

Similar sorts have been done with several times 10^9 cells containing several times 10^8 labelled cells (data not shown). At present the largest column of the MACS system can retain at most approximately 5×10^8 cells.

Enrichment

The experiment from which histograms shown in Figure 5 were derived was optimized for simultaneous purification of labelled and unlabelled cells by extensive washing of the column before elution of positive cells. A total of 1.9×10^7 murine spleen cells were labelled with biotinylated anti-kappa light chain antibodies (4), staining about 95% of all B lymphocytes, streptavidin-fluorescein, and superparamagnetic beads. The cells were sorted on a column of 3 ml volume, collecting the negative cells in 20 ml PBS/BSA/azide. After washing with 20 ml PBS/BSA in the magnetic field, the positive cells were eluted following removal of the magnet, applied a second time to the magnetized column, washed again with 50 ml/25 min. and finally eluted without the magnetic field. Both washes together contained about 10% of the total cells, mostly unlabelled (data not shown). Before separation 65.5% of the cells were in the counting window for negative cells and 34.5% of the cells in the window for positive cells (Fig. 5a). After MACS separation the unretained fraction contained 97.9% nonfluorescent and 2.1% weakly positive cells, recovering about 85% of the unlabelled cells (Fig. 5b). The retained fraction, eluted from the MACS column, contained 98.5% of fluorescently labelled and only 1.5% of unlabelled cells, with a recovery of more than 90% of the positive cells (Fig. 5c). The enrichment of positive cells was 124-fold. Throughout the sorting procedure the cells retained their overall viability of more than 90%.

MACS Enrichment for FACS

Small subpopulations of labelled cells cannot be isolated directly by MACS. However, the enrichment of small subpopulations of magnetofluorescently stained cells by MACS may facilitate analysis and isolation of such subpopulations by fluorescence-activated cell sorting. Due to the magnetic and fluorescent double-labelling, MACS derived cells, either depleted or enriched, can be used immediately in flow cytometry, for which additional fluorescent markers can be easily introduced either before, during, or after magnetic sorting.

Enrichment of rare cells for FACS is demonstrated by the data shown in Figure 6. J558L-MK-F cells, expressing H-2K^k antigens (20), were mixed to J558L cells not expressing this antigen. The cell mixture was stained with biotinylated monoclonal anti-H-2K^k antibody 116-22 (20), streptavidin-fluorescein, and superparamagnetic beads. Counting at the gate indicated, 0.28% of the cells were positive (Fig. 6a), although this fraction still may have contained negative J558L cells, trailing from the negative peak. A total of 1.6×10^8 cells were applied to a ferromagnetic column of 0.3 ml volume with a capacity of $\sim 10^7$ cells. This small column was deliberately chosen in order to minimize background problems. Non-adherent cells were washed off extensively at a flow rate of ~1 ml/min with more than 20 ml of PBS/BSA/azide. The negative fraction was largely depleted of stained cells, containing only 0.07% of positive cells (Fig. 6b). After removal of the magnetic field, 10^6 cells were eluted from the column. This fraction (Fig. 6c) contained 14.4% positive cells. This corresponds to an enrichment of 60-fold and a recovery of 63%.

DISCUSSION

The concept of MACS combines quantitative and specific labelling of cells with fluorescence and superparamagnetic beads. Labelled cells are efficiently retarded in high-gradient magnetic fields generated in magnetized ferromagnetic steelwool and can be recovered easily and viably from the steelwool after demagnetization.

The magnitude and direction of the force F on a mag-

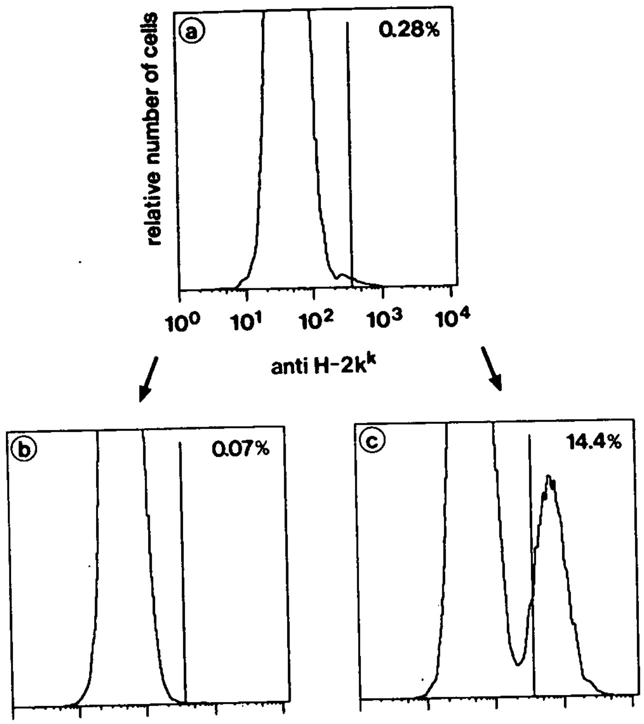

FIG. 6. MACS enrichment for FACS. Histograms of J558L-MK-F cells, expressing an H-$2K^k$ gene, and mixed to J558L cells not expressing this gene, stained with a biotinylated monoclonal anti H-$2K^k$ antibody, streptavidin-fluorescein, and superparamagnetic beads. As analysed by FACS/EDESK, before separation by MACS (a) 0.28% of the cell mixture were in the window set for counting positive cells. The MACS depleted population (b) contains very few if any positive cells. The retained and eluted fraction (c) is enriched to 14.4% for labelled cells. Live cells had been gated according to forward and side scatter and propidium iodide fluorescence and are displayed as in Figure 4.

netically labeled cell is dependent on the gradient of the magnetic field (grad $|B|$) and the number of magnetic moments inducable on a labelled cell:

$$F = N\,S\,\mu_B\,\mathrm{grad}\,|B|$$

where S is the number of Bohr magnetons (μ_B) per bead and N is the number of beads per cell.

For use of beads with small S, we constructed a compact separation unit for the generation of high magnetic fields. This approach, called high gradient magnetic separation (HGMS), has been used before in the mining and china clay industries and also for separation of cells. Red blood cells can be made paramagnetic (9) and retained in a HGMS column. Paramagnetic red blood cells conjugated to protein A or paramagnetic ferritin have been used to label other cells specifically and separate them from a cell mixture (16,17). High magnetic fields (4 Tesla), generated in superconducting magnets, are then required for separation (18,19).

A ferromagnetic high-gradient system allows generation of extremely high magnetic forces and rapid demagnetisation. Small ferromagnetic structures, such as steelwool wires, placed in a magnetic field, disturb this field, producing high magnetic gradients and generating in their immediate neighborhood magnetic forces of some 10^4 T/m compared to some 10 T/m in conventional geometries. Another advantage of the MACS geometry is that in a column of steelwool, a magnetised cell has to be moved only over a distance of 100–200 μm to the next wire compared to some mm in a macroscopic setup, using a magnet for separation of cells in culture dishes or tubing.

A further increase in quality of separation by MACS comes from another, yet undescribed effect. In a magnetic field in the neighborhood of a ferromagnetic wire a macroscopic flow is induced if the solution around the wire contains magnetic particles. This can be viewed as a phase transition, the "magnetic fluid" in the vicinity of the wire replacing the "nonmagnetic solution". Due to this effect all cells are transported next to a wire, where the magnetic gradient is highest. Also weakly magnetised cells will bind to the wire then. To protect the ferromagnetic matrix from corrosion, stabilize the matrix, remove "flow-holes," and smoothen the surface of the wires for the cells, we developed a method to coat the steelwool-wires with plastic.

Compared to paramagnetic beads, superparamagnetic microbeads (e.g., small magnetite crystals) have a much stronger magnetisation capacity. Several small ferrit-containing beads for immunospecific cell separation have been described (6,12). For the MACS system we have used biotinylated ferrit-dextran beads. Ferrit was precipitated with sodium hydroxide out of a solution of Fe^{2+}, Fe^{3+} ions, and dextran. The efficiency of generation of beads was improved by titrating the base slowly to the mixture of Fe ions. HGM separation for purification of the microbeads was used rather than gel-chromatography because nonmagnetic beads and beads with low ferrit content are eliminated. No further concentration steps are required. The beads consist of irregular dextran aggregates with trapped ferrit microcrystals.

Many thousands of the superparamagnetic beads (diameter$_{particle}$ ≤ 200 nm) would fit on the surface of an average lymphocyte, thereby allowing a quantitative staining similar to that obtained by antibodies. Staining kinetics are those of macromolecules; i.e., staining is complete within a few minutes. In the context discussed here, the beads are individually invisible in light microscope and flow cytometer and thus don't interfere with the optical analysis of the cell they are bound to. The beads can be sterilized by filtration. The small magnetic moment of the superparamagnetic beads used here is of advantage for keeping them in a stable solution even in a magnetic field and, most important, for elution of labelled cells from a demagnetized ferromagnetic matrix. In the cellular systems analysed up to now, e.g., stimulation of murine B lymphocytes with the mitogen LPS (unpublished results) or human B lymphocytes with the mitogen SAC (Abts et al., submitted) the labelling did not interfere with

viability or in vitro differentiation of the B cells into plasmablasts.

The specific labelling with biotinylated antibodies, streptavidin-fluorochrome conjugates, and biotinylated superparamagnetic beads follows the general rules for immunological staining. The reagents should be titrated, and in general small amounts will be sufficient. The success of the MACSorting is dependent on the quality of the immunofluorescent staining. In general the sensitivity of MACS is such that populations discrete in flow analysis can be separated by MACS as well. Background staining due to problems with the antibody and/or avidin may lead to adsorbance of the unspecifically stained cells on the magnetic column. Frequently this will be the case for dead cells, which stain with avidin-conjugates and superparamagnetic beads and are retained on the column. In depletion experiments this effect can be used to increase the viability of the negative fraction. If the positive fraction is wanted as well, dead cells could be removed using Ficoll-Paque prior to magnetic separation or by a mock magnetic separation staining the cells without antibody but with streptavidin and superparamagnetic beads.

The quality of MACSorting depends not only on the immuno-magnetic staining, but also on several physical parameters of the separation, i.e., number of labelled cells, capacity and consistency of the ferromagnetic matrix, speed and volume of washing.

For depletion of labelled cells, columns with ample capacity, at least for the *total* number of cells to be sorted, and slow flow rates of less than 1 volume of column/min for washing are optimal. Thus virtually every labelled cell will be retained on the column and depletion rates of several thousendfold can be achieved (Fig. 4) with more than 90% recovery. Moreover, by MACS the negative fraction is depleted of dead cells and the negative cells are not touched by any reagent.

For isolation of labelled cells, columns with limiting capacity are preferable, i.e., columns that can retain little more than the number of *labelled* cells. Negative cells are removed by slow washing (see above) as a rather pure population, before losely attached cells, negative and positive, are washed off at high flow rate, e.g., several column volumes/min. Few cells are lost in this step. Finally the positive cells are eluted after removal of the magnetic field at the highest possible flow rate. The cells are detached from the wires by flushing the column with PBS/BSA and can be collected in a small volume. Enrichment rates of more than hundredfold can be achieved (Figs. 5,6). This will be sufficient for most applications, aiming at rather pure preparations of substantial subpopulations, e.g., separating B and T lymphocytes from lymphoid organs.

The isolation of rare cells, i.e, subpopulations below 1%, to homogeneity will be difficult by MACS alone. In this situation, however, MACS will be an ideal complement to other cell separation methods, like micromanipulation or FACS, because the rare cells are already fluorescently labelled and considerably enriched (60-fold in the experiment shown in Fig. 6). Thus by parallel magnetic sorting the initially large cell numbers are reduced and rare cells enriched for serial multiparameter sorting methods. With respect to FACS, preenrichment by MACS has several additional advantages: The subpopulation of rare cells may become more clearly visible, thus facilitating the setting of sort windows (compare Fig. 6a and c), low cell numbers allow low flow rates which minimize the amount of sort-abort signals and low cell numbers also shorten sorting time. In an experiment like the one described in Figure 6, the sorting of initially 1.8×10^8 cells (Fig. 6a) would have taken about 10 h continuous sorting at 5,000 cells/s. Sorting the 10^6 cells enriched by MACS (Fig. 6c) at a flow rate of 1,000 cells/s could be completed in 20 min. Including the additional time required for MACSorting, about 30 min, the experiment would take about 1 h with MACS rather than 10 without, a gain that has profound effects on viability of the cells and happiness of the experimenter.

In summary, the MACS system described here provides a simple, cheap, powerful, and convenient parallel cell separation technology for routine use in cell laboratories. The specific labelling with biotinylated antibodies, streptavidin-fluorochrome conjugates, and biotinylated superparamagnetic beads allows immediate control of the magnetic separation by fluorescence-microscope or flow cytometer and further processing by FACS. We did not yet take full advantage of the quantitative character of the magnetic labelling, which would be a challenge for the future.

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